

5 What is claimed is:

1. A competitive method for estimating the concentration in a sample of a *Bacillus anthracis* protein selected from the group consisting of protective antigen (PA), lethal factor (LF) and edema factor (EF) said competitive method selected from the group consisting of fluorescence polarization (FP), fluorescence lifetime (FLT) and fluorescence resonance energy transfer (FRET) the said competitive
10 method comprising the steps:
 - a. intermixing a said sample suspected of containing said protein with a specific antibody to said protein and a competitive reagent labeled with a fluorochrome capable of binding to said specific antibody to produce a
15 mixture;
 - b. incubating said mixture for 15 seconds to 5 minutes;
 - c. detecting binding interaction of said protein and antibody.
2. The method of claim 1, wherein said detection is by change in fluorescence polarization.
- 20 3. The method of claim 1, wherein said detection is by change in fluorescence life-time.
4. The method of claim 1, wherein said detection is by sensitized fluorescence of the acceptor or by quenching of donor fluorescence or by fluorescence depolarization.
5. The method of claim 1, wherein said method comprises the additional steps of:
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 - d. measuring the fluorescence polarization of a negative control solution of said fluorochrome-labeled competitive reagent, a positive control solution

- 5 of said fluorochrome-labeled competitive reagent exposed to a known
 amount of said protein, or both, and
- e. comparing the measured fluorescence polarization of said mixture or said
 antibody detection mixture with the measured fluorescence polarization of
 said negative control solution, said positive control solution, or both.
- 10 6. The method of claim 1, wherein said competitive reagent is native or recombinant
 Bacillus anthracis protein or fragments of said protein.
7. The method of claim 1, wherein said protein can be detected down to less than 5
 μg/ml or 6 nM within 5 minutes.
8. The method of claim 1 wherein said sample is selected from the group consisting
15 of broth culture media of growing *Bacillus anthracis* or bodily fluids.
9. The method of claim 1, wherein said incubation step (b) occurs in less than 30
 seconds for concentrated samples.
10. The method of claim 1 wherein said incubation step (b) occurs in 4 to 5 minutes
 for low concentration samples.
- 20 11. The method of claim 1 wherein said fluorochrome is pH independent.
12. The method of claim 1 wherein said fluorochrome is selected from the group
 consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa
 568, Alexa 594, Aminonaphthalene, Benzoxadiazole, BODIPY 493/504, BODIPY
 505/515, BODIPY 576/589, BODIPY FL, BODIPY TMR, BODIPY TR,
25 Carboxytetramethylrhodamine, Cascade Blue, a Coumarin, Cy2, CY3, CY5,
 CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium Homodimer II,
 Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted

5 mutants T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258,
IAEDANS, an Indopyras Dye, a Lanthanide Chelate, a Lanthanide Cryptate,
Lissamine Rhodamine, Lucifer Yellow, Maleimide, MANT, MQAE, NBD,
Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin, a
Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide,
10 Pyridyloxazole, Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas
Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12, flavin-adenine
dinucleotide, and nicotinamide-adenine dinucleotide.

13. The method of claim 1 wherein said fluorochrome concentration is 1 nM or less
and the sample millipolarization is increased or decreased by at least 10 mP.

15 14. The method of claim 1 wherein the said antibody is polyclonal or monoclonal.

15. The method of claim 8, wherein said bodily fluids are selected from the group
consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal
transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool,
gastric fluid, synovial fluid, phlegm, and other clinical and laboratory specimens
20 and samples.

16. A competitive method for estimating the concentration of specific antibody in a
sample of bodily fluids to a protein from *Bacillus anthracis* selected from the
group consisting of protective antigen or PA, lethal factor or LF and edema factor
or EF said competitive method selected from the group consisting of fluorescence
25 polarization or FP, fluorescence lifetime or FLT and fluorescence resonance
energy transfer or FRET the said competitive method of claim comprising the
steps:

- 5 a. intermixing a said sample suspected of containing said antibody with a
 competitive reagent labeled with a fluorochrome capable of binding to
 said specific antibody to produce an antibody detection mixture;
- b. incubating said antibody detection mixture for 15 seconds to 5 minutes;
- c. detecting binding interaction between said protein and antibody.
- 10 17. The method of claim 16, wherein said detection is by change in fluorescence
 polarization.
18. The method of claim 16, wherein said detection is by change in fluorescence life-
 time.
19. The method of claim 16, wherein said detection is by sensitized fluorescence of
15 the acceptor or by quenching of donor fluorescence or by fluorescence
 depolarization.
20. The method of claim 16, wherein said method comprises the additional steps of:
- d. measuring the fluorescence polarization of a negative control solution of
 said fluorochrome-labeled competitive reagent, a positive control solution of
20 said fluorochrome-labeled competitive reagent exposed to a known amount of
 said antibody, or both, and
- e. comparing the measured fluorescence polarization of said mixture or said
 antibody detection mixture with the measured fluorescence polarization of
 said negative control solution, said positive control solution, or both.
- 25 21. The method of claim 16, wherein said incubation step (b) occurs in less than 30
 seconds for concentrated samples.

- 5 22. The method of claim 16 wherein said incubation step (b) occurs in 4 to 5 minutes
for low concentration samples.
23. The method of claim 16 wherein said fluorochrome is pH independent.
24. The method of claim 16 wherein said fluorochrome is selected from the group
consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa
10 568, Alexa 594, Aminonapthalene, Benzoxadiazole, BODIPY 493/504, BODIPY
505/515, BODIPY 576/589, BODIPY FL, BODIPY TMR, BODIPY TR,
Carboxytetramethylrhodamine, Cascade Blue, a Coumarin, Cy2, CY3, CY5,
CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium Homodimer II,
Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted
15 mutants T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258,
IAEDANS, an Indopyras Dye, a Lanthanide Chelate, a Lanthanide Cryptate,
Lissamine Rhodamine, Lucifer Yellow, Maleimide, MANT, MQAE, NBD,
Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin, a
Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide,
20 Pyridyloxazole, Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas
Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12, flavin-adenine
dinucleotide, and nicotinamide-adenine dinucleotide.
25. The method of claim 16 wherein said fluorochrome concentration is 1 nM or less
and the sample millipolarization is increased or decreased by at least 10 mP.
- 25 26. The method of claim 16, wherein specificity of detection is 96 – 99%.
27. The method of claim 16, wherein said bodily fluids are selected from the group
consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal

5 transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool,
gastric fluid, synovial fluid, phlegm, and other clinical and laboratory specimens
and samples.

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